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Grazing activity and ruminal bacterial population associated with frothy bloat in steers grazing winter wheat

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ABSTRACT

Two grazing experiments were designed to elucidate the shifts in rumen bacterial populations (Exp. 1) and grazing activities (Exp. 2) in wheat forage diet between bloated and nonbloated steers. In Exp. 1, the bacterial DNA density was greatest for Ruminococcus flavefaciens, Streptococcus bovis, and Eubacterium ruminantium among tested strains when steers were fed bermudagrass hay (d 0). Steers that grazed wheat forage, however, increased the bacterial density of 6 major rumen bacterial populations in bloated steers, indicating that frothy bloat may be associated with a species-specific bacterial population. In Exp. 2, overall time, total grazing, and ruminating time did not differ between bloated and nonbloated steers. In contrast, idling time was greater for bloated ($P < 0.01$) than for nonbloated steers (10.9 vs. 7.9 h/d, respectively). Bloated steers did not differ in total grazing activity patterns; however, grazing activity in bloated steers decreased ($P < 0.05$) from 0400 to 0700 h and 1400 to 1800 h. Ruminating activity in nonbloated steers peaked from 0200 to 0500 h and 1900 to 2200 h but was lower ($P < 0.05$) for bloated than for nonbloated steers from 0100 to 0600 h and 0700 to 1200 h. The data suggest that rumen bacterial populations and grazing activities changed when steers experienced frothy bloat.

Key words: bacterial population, bloat, grazing activity, steer, wheat forage

INTRODUCTION

The frequency and severity of frothy bloat in cattle grazing wheat pasture apparently result from complex interactions among management, plant, and animal as modified by ambient environmental conditions (temperature, solar radiation, and dew or frost; Majak et al., 2003; Min et al., 2005a,b; Pinchak et al., 2005). Although total and soluble plant proteins are known to be considered primary bloat precursors (Bartley et al., 1975), little is known about how these factors change in steers grazing wheat forage. Bloat expression is likely in response to the rate and amount consumed and ruminal availability of plant bloat cursors (Howarth et al., 1991; Majak et al., 2003). The design of this study aimed to measure grazing activity patterns and selected ruminal bacterial populations as an integrated indicator of grazing cattle response to frothy bloat. This unique approach was based on eructation inhibition when the rumen-cardia is foam covered (Dougherty, 1953; Cole and Boda, 1960). Associated intraruminal pressures increase up to 70 mm Hg or 9.34 kPa (Lippke et al., 1972) leading to eructation cessation. The hypothesis of this experiment was that increased intraruminal pressures resulted in postingestive malaise (Provenza, 1995; Phy and Provenza, 1998) that would manifest itself in altered daily and diurnal activity patterns. Sowell et al. (1999) clearly documented decreases in feeding and watering bouts between healthy and morbid feedlot cattle. There are no published studies on the effect of frothy bloat on grazing activity and ruminal bacterial population. Rutter et al. (2004) speculated that grazing activity would be altered by bloat in

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grazing cattle. Our primary objective was to quantify differences in diurnal grazing patterns and 7 dominant ruminal bacterial populations between bloated and nonbloated steers grazing wheat forage. The secondary objective was to elucidate the effect of wheat growth stage on diurnal grazing activity.

MATERIALS AND METHODS

Animals and Experimental Design

All procedures related to the animals used in the current study were accepted by the Texas A&M University Animal Use and Care Committee, and the animals were cared for according to its guidelines. Two grazing experiments were designed to elucidate the shifts in rumen bacterial populations (Exp. 1) and grazing activities (Exp. 2) in wheat forage diet between bloated and nonbloated steers. Research was conducted on continuously cropped wheat pasture in Wilbarger county, Texas (33°57′N, 99°26′W).

Exp. 1: Measurement of Bacterial Population

For Exp. 1, 6 ruminally cannulated steers (Angus × Hereford × Brangus; initial BW = 375 ± 30 kg) were used and grazed at an average forage allowance of 14 kg of DM/100 kg of BW per day during vegetative (January to February) and reproductive (March to April) stages of growth. The experiment was a qualitative study to assess 7 dominant ruminal bacterial populations (*Streptococcus bovis* strain 26, *Prevotella ruminicola* strain 23, *Eubacterium ruminantium* B1C23, *Fibrobacter succinogenes* ssp. S85, *Ruminococcus flavefaciens* C94, *Selenomonas ruminantium*, and *Ruminobacter amylophilus*) associated with frothy bloat (bloated vs. nonbloated) when grazing winter wheat forage.

In February 18 through April 20, steers were visually monitored daily (at 0800 h) and scored for bloat (0 = no bloat, 3 = severe bloat; Paisley and Horn, 1998). Overall (n = 3) mean bloat scores were <2 as a result of low bloat severity. Rumen samples were collected from 6 steers after 1 mo on a bermudagrass hay (d 0) for each animal. Steers were then transferred to wheat pasture in February and allowed to graze wheat forage for 70 d before mid-February, coinciding with the time of greatest incidence in frothy bloat in north Texas. Ruminal contents (~500 g/steer) were collected from the 6 steers on d 50 and 70 for analysis of bacterial populations associated with frothy bloat. A pure sward of winter wheat forage was used under continuous grazing during the experimental period. A pure sward of the vegetative stage of fresh wheat forage was managed under ad libitum access (18 kg of DM/100 kg of BW per d) during the experimental period (Pinchak et al., 1996). All steers were provided ad libitum access to a free choice of mineral supplement (ACCO Wheat Advantage Mineral, Minneapolis, MN) and water.

DNA Extraction

Genomic bacterial DNA was isolated from 1 mL of each unknown rumen sample according to the method described in the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA). Concentrations of DNA were measured using a NanoDrop Spectrophotometer (ND-1000, Wilmington, DE). The primers designated to detect the target species (Tajima et al., 2001) are listed in Table 1. The PCR amplifications were conducted using species-specific PCR primers. To minimize animal-to-animal variations, the aliquots of rumen fluid from 4 animals were mixed after DNA extraction.

Microorganism and PCR

A set of PCR primers was designed and validated (Tajima et al., 2001) for specific detection of *Streptococcus bovis* strain 26, *Prevotella ruminicola* strain 23, *Eubacterium ruminantium* B1C23, *Fibrobacter succinogenes* S85, *Ruminococcus flavefaciens* C94, *Selenomonas ruminantium* strain JCM 6582, and *Ruminobacter amylophilus* strain ATCC29744. Dr. J. Yanke, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, generously provided these organisms. In this experiment, 7 ruminal bacteria were chosen because these strains are known to be major proteolytic, amylolytic, and cellulolytic bacteria in the rumen of cows.

Strain purity was confirmed by determining single colony morphology on agar plates and by a single consistent cellular morphology in liquid cultures examined under the microscope (J. Yanke, 1999, Agri-Food Canada, PO Box 3000, Lethbridge, Alberta, CA, personal communication). All isolates were inoculated into anaerobic basal medium in Hungate tubes for 24 h at 39°C from their respective long-term storage vials (lyophilization). Lyophilized bacterial isolates were reinoculated into anaerobic plant protein medium and incubated for 24 h at 39°C.

The PCR amplifications were performed on the PTC-200 Peltier Thermal Cycler (MJ Research Inc., Waltham, MA) with the following program: 1) denaturation at 95°C for 3 min; 2) subsequent 35 denaturing cycles at 95°C for 30 s; 3) various annealing temperatures (described in Table 1) for 30 s and extension at 92°C for 1 min (Tajima et al., 2001). Primers [50 pmol of each per reaction mixture; primer 2 and primer 3 (Integrated DNA Technologies Inc., Coralville, IA); Sheffield et al., 1989; Muyzer et al., 1993] were mixed with Jump Start Red-*T*aq Ready Mix (Sigma Chemical Company, St. Louis, MO), according to the kit instructions, 250 ng of template DNA from rumen digesta of pooled steers, and 5% (wt/vol) acetamide to eliminate preferential annealing (Reysenbach et al., 1992). The PCR products were separated by electrophoresis on a 2% precast agarose E-gel system (Invitrogen, Carlsbad, CA).

The 16S rDNA from ruminal DNA isolated from the rumen of steers fed either bermudagrass hay or grazing winter wheat forage associated with bloat severity was PCR amplified us-

Table 1. The PCR primers for detection of rumen bacteria

Target bacteria	Primer[1]	Annealing temperature (°C)	Product size (bp)
Fibrobacter succinogenes S85	F: GGTATGGGATGAGCTGC R: GCCTGCCCTGAACTATC	62	445
Ruminococcus flavefaciens strain C94	F: GGACGATAATGACGGTACTT R: GCAATCTGAACTGGGACAAT	62	835
Streptococcus bovis strain 26	F: CTAATACCGCATAACAGCAT R: AGAAACTTCCTATCTCTAGG	57	869
Prevotella ruminicola strain 23	F: GGTTATCTTGAGTGAGTT R: CTGATGGCAACTAAAGAA	53	485
Eubacterium ruminantium B1C23	F: GCTTCTGAAGAATCATTTGAAG R: TCGTGCCTCAGTGTCAGTGT	57	671
Selenomonas ruminantium JCM6582	F: TGCTAATACCGAATGTTG R: TCCTGCACTCAAGAAAGA	53	513
Ruminobacter amylophilus ATCC29744	F: CAACCAGTCGCATTCAGA R: CACTACTCATGGCAACAT	57	642

[1]Each primer was purchased from Integrated DNA Technologies Inc., Coralville, Iowa. F = forward; R = reverse.

ing primers, and the resulting products were separated on 2% agarose E-gel (Figure 1). Density signal of DNA was judged by the density signals based on the brightness, wideness, and size of the bands.

Exp. 2: Measurement of Grazing Activity

For Exp. 2, 11 castrated steers (initial BW = 320 ± 20 kg) were used and grazed at an average forage allowance of 14 kg of DM/d per 100 kg of BW during the experimental periods (February to March). Cattle were adapted to grazing wheat for 45 d and to being fitted with grazing activity monitors and harnesses for 14 d before initiation of the grazing activity experiment. Paddocks (14.1 ha) were continuously grazed over the grazing period. The effect of bloat status and grazing activity of a subset of 6 bloated and 5 nonbloated steers was examined on wheat forage swards maintained at 8 to 15 cm sward surface height. Forage allowance was offered at that sufficient to promote bloat (Min et al., 2005a) and sustain acceptable animal performance (Pinchak et al., 1990, 1996). At the time grazing activity was measured, 3 hand-clipped forage samples (about 500 g) were collected from random locations in the paddock for nutritive value analyses.

During the experimental period, relative grazing and ruminating activity dynamics for each steer (bloated vs. nonbloated steers) over 24-h periods were recorded automatically using jaw-movement sensors (Ultra Sound Advice; IGER, North Wyke, Okehampton, Devon, UK) to measure grazing, ruminating, and idling activity (Rutter, 2000a). In addition, the number of jaw movements, grazing, and ruminating bouts were continuously recorded and stored in an onboard data logger. Periods with no jaw movements or unidentified movements were classified as "other activities." Recordings were analyzed using a peak recognition algorithm with a noise threshold capable of differentiating between periods of grazing and ruminating activity, and grazing jaw movements (Rutter et al., 1997; Rutter, 2000b). There was an incomplete 24-h collection from a bloated steer that was omitted from further analyses.

Chemical Analysis

Three hand-clipped forage samples were subsequently pooled, thoroughly mixed, dried in a forced-air oven at 60°C for 48 h, and ground (Cyclone Sample Mill, UDY CO., Fort Collins, CO) to pass 1-mm sieve for CP, NDF, ADF, and IVDMD analyses. The CP from wheat forage samples was determined by the Kjeldahl digestion procedure (AOAC, 1990). The NDF, ADF, and IVDMD of dried forage samples were determined using the Filter Bag Technique (ANKOM Technology Corp., Fairport, NY).

Statistical Analysis

Data were analyzed as a repeated-measures analysis using the MIXED procedures of SAS (SAS Institute Inc., Cary, NC). Data are presented as least squares means and the associated SEM. The variables in the present experiment included 7 dominant rumen bacterial populations associated with bloat in steers grazing winter wheat. The model includes the relationship of frothy bloat in cattle fed bermudagrass hay and grazing wheat forage to changes in 7 dominant ruminal bacterial populations. Animals were experimental units and were treated as a random effect. To minimize animal-to-animal variations, the aliquots of rumen fluid from 3 animals were mixed after DNA

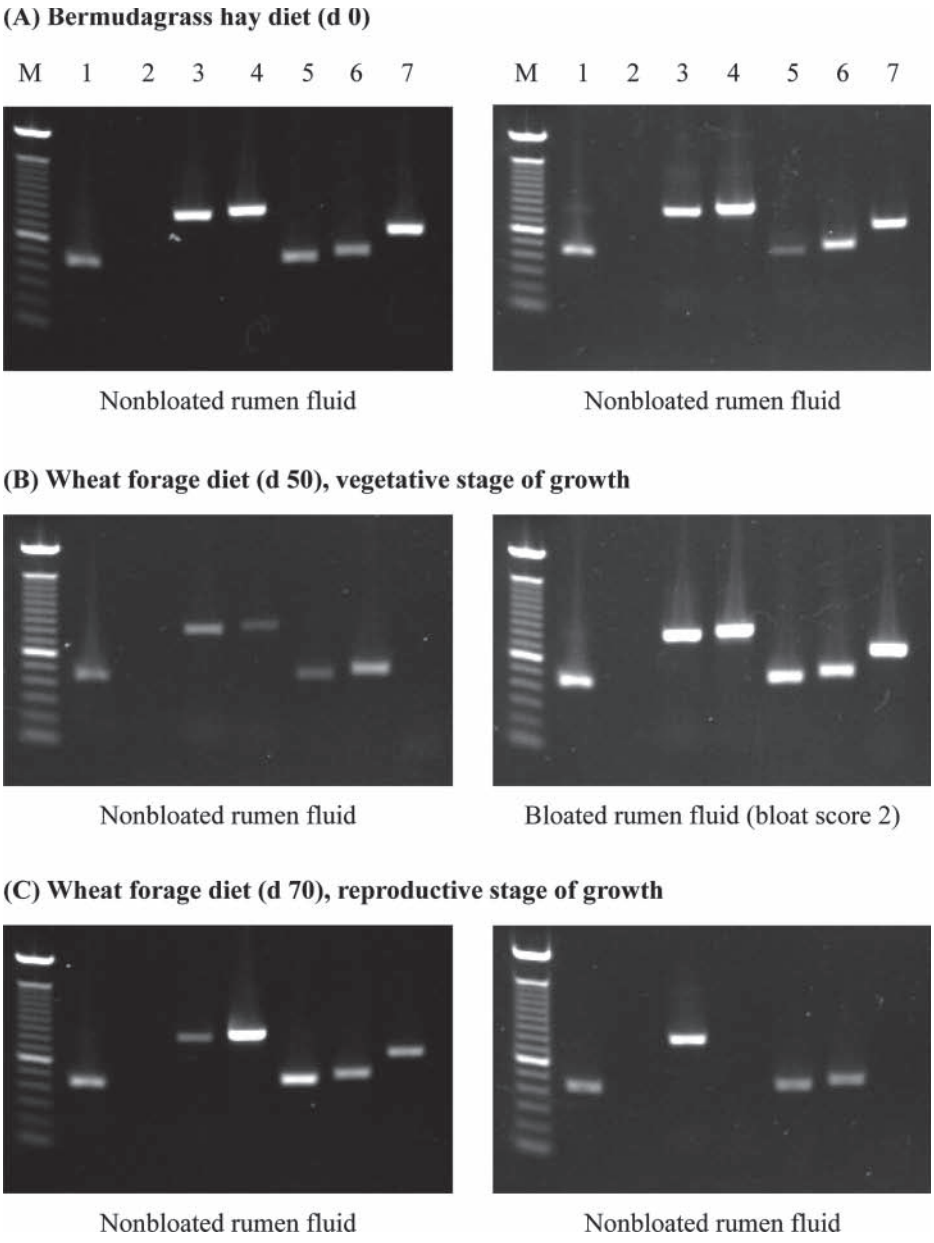

Figure 1. Qualitative PCR detection of 7 ruminal bacterial populations in the rumens of steers (n = 3) for which the diet and bloat frequency had been changed from bermudagrass hay diet to wheat forage diet. Lane M, DNA size marker. Lanes: 1, *Fibrobacter succinogenes* ssp. S85; 2, *Ruminobacter amylophilus*; 3, *Ruminococcus flavefaciens* C94; 4, *Streptococcus bovis* strain 26; 5, *Prevotella ruminicola* strain 23; 6, *Selenomonas ruminantium*; 7, *Eubacterium ruminantium* B1C23.

extraction; hence, only the phenotypic visual analysis per each strain on each time point is reported (Min et al., 2012).

The grazing activities and temporal patterns of each activity by 1-h time steps within 24 h were analyzed by ANOVA using the MIXED procedure of SAS. Animals were the experimental unit and were treated as a random effect. Mean separation was performed using least significant differences when the F statistic was significant ($P < 0.05$). Variables included forage nutrient contents (DM, CP, NDF, ADF, and IVDMD), grazing activities (grazing time, ruminating, idling, and others), jaw movements, mastication, prehension, boli, and bouts. The grazing activities, ruminating, mastication, prehension, boli, and idling time were examined between physiological state (bloated vs. nonbloated) during 24 h. The model included bloat status (bloated vs. nonbloated steers), grazing activities, and associated interactions.

RESULTS AND DISCUSSION

Exp. 1: Nutritive Composition of Wheat Forage

The mean DM, CP, NDF, ADF, and IVDMD of the pasture samples are shown in Table 2. Plant DM and ADF were similar between forage sampling periods. Total CP concentration ($P < 0.001$) was greater, IVDMD and ADF unchanged, and NDF concentration less ($P < 0.01$) between the sampling time periods in wheat forage. This finding is in agreement with a previous study (Pinchak et al., 1996).

Bacterial Populations Associated with Bloat Dynamics

Previous research reported that in rumen-fistulated Holstein cows fed hay-basal diet, 90.2% of DNA sequences belonged to the low guanosine + cytosine (G+C) gram-positive bacteria (e.g., *R. flavefaciens* and *R. albus*) phylum with the minor inclusion of the *Cytophaga-Flavobacter-Bacteroides* (3.9%), *Protobacteria* (3.9%), and high G+C gram-positive bacteria (3%; Tajima et al., 2000). In this experiment, the bacterial density was greatest for *R. flavefaciens* (3), *S. bovis* (4), and *E. ruminantium* (7) among tested strains when steers fed a bermudagrass hay diet (Figure 1A; d 0).

Steers grazed wheat forage, however, increased the bacterial density of 6 major rumen bacterial populations in bloated steers, indicating that frothy bloat may be associated with a species-specific bacterial population. Of the 7 strains evaluated, no signal on *R. amylophilus* was ever detected in either bloat class of steers on any diet (Figures 1 A, B, and C); this absence, may have resulted from very low population densities because

Table 2. The effect of sampling time of wheat forage on forage nutrient content and IVDMD of winter wheat forage during 1st (February 14 to 28) and 2nd sampling periods (March 28 to 31), Vernon, Texas

Item (% of DM)	n[1]	CP	NDF	ADF	IVDMD
February	4	29.5	43.3	26.7	95.1
March	4	20.8	46.2	28.7	93.0
SEM		1.39	0.69	1.67	1.02
P-value		0.001	0.01	0.27	0.24

[1]n = sample size.

these steers from birth through the end of the experimental period had consumed forage-only diets containing limited or no α-linked glucose molecules (maltose, maltodextrins, and starch) required as substrate by *R. amyolphilus* (Anderson, 1995). However, bloated and nonbloated steers in the bermudagrass feeding phase (Figure 1A) had all the remaining 6 strains present.

During the peak bloat period (Figure 1B; d 50) and rapid vegetative growth phase, the density signals for 6 strains were greater in bloated than in nonbloated steers. In the bloated group (d 50) during the vegetative stage of growth, DNA density signals of 6 bacterial strains (*F. succinogenes*, *R. flavefaciens*, *S. bovis*, *P. ruminantium*, *S. ruminantium*, *E. ruminantium*) increased during the onset of bloat in steers grazing high-quality wheat forage diet compared with the bermudagrass (d 0) or late growth stage of wheat forage (d 70) diet. The relatively large density signal of *E. ruminantium* (7) present in the bermudagrass feeding phase was completely absent in the peak bloat phase (d 50) in the nonbloated steer. Plant soluble protein in wheat forage is known to be rapidly digested by ruminal microorganisms, which results in the production of large volumes of ruminal gasses, a precursor of frothy bloat (Clarke and Reid, 1974; Min et al., 2005a). The shifts observed in rumen bacterial species are associated with differences in G+C-containing species between bloated and nonbloated steers and implicates major alterations in microbial populations associated with pasture bloat severity (Fletcher and Hafez, 1960; Min et al., 2006). Research has reported that the bio-film fractions from ruminal digesta produced more during the onset of bloat in steers fed a high-grain ration (Gutierrez et al., 1959) or high-quality forage diets (Ladino clover or wheat forage; Gutierrez et al., 1963; Min et al., 2006, respectively). A probable source of bio-film precursors is cytoplasmic granules containing polysaccharides that frequently occur in rumen bacteria such as *Megasphaera elsdenii* (Brown et al., 1975), *R. albus* (Cheng et al., 1977), *Selenomonas ruminantium* (Wallace, 1980), *S. bovis* (Cheng et al., 1976), and mixed rumen bacterial cells from the rumen of cattle fed a high-energy diet (Cheng et al., 1973, 1976; Russell, 1998). Recently, Min et al. (2006) reported that some rumen microbial strains produced more bio-film than do others. This is supported by our in vivo PCR assay that overall 6 bacterial populations have been shown to increase during the onset of bloat in steers grazing high-quality wheat forage. These relationships could suggest that wheat pasture bloat may be caused by increased production of bio-film (Min et al., 2006) as a result of diet-influenced shifts in the rumen bacterial population associated with soluble carbohydrate production during the peak bloat period.

Overall data found in Exp. 1 imply that wheat pasture bloat may be related to different responses in rumen bacterial populations between bloating and nonbloating animals when diet composition was changed. Further research is required to define what kind of ruminal symbiotic systems promote frothy bloat in the rumen of cattle grazing wheat pasture and the relationships of rumen microorganisms to seasonal patterns, forage chemical composition, intake rate, and grazing behavior.

Exp. 2. Bloat Effects on Grazing Activity of Steers

On a daily basis (Table 3), grazing time and ruminating time numerically but not statistically differ between bloated and nonbloated steers. In contrast, idling time was 28% greater ($P < 0.001$) for bloated than for nonbloated steers (10.9 vs. 7.8 h per 24 h). The exact nature of idling time estimated with the IGER systems under wheat pasture grazing conditions is as yet unknown. Noningestive and nonruminating activities could reflect potential satiety in nonbloated steers (Provenza, 1996), whereas idling time by bloated steers may suggest bloat-induced malaise (Provenza, 1996).

Further separation of daily activity patterns showed that total jaw movements were 20% less ($P < 0.01$; 52,653 vs. 65,872 jaw movements) in bloated than in nonbloated steers. However, there was no difference in ruminating bouts. On a daily basis, these responses would collectively support a hypothesis that bloat disrupts grazing-related activities and processes and not those associated with ruminating. This is the first report of bloat decreasing grazing activity but increasing idling time in wheat-pasture steers. Majak et al. (2003) reported that ruminal movements usually increase in the early stages of bloat but decrease and even completely cease when ruminal distension is extreme. Results from a previous review reported that bloated animals increased intrarumen and blood pressures but decreased oxygen use (Colvin and Backus, 1988), which would support the decreased grazing activity and increased idling activity found in this experiment.

There was no difference in mastication jaw movements between bloated

Table 3. The effect of physiological state (nonbloated vs. bloated steers) on relative grazing time, ruminating time, idling time, jaw movements, and bouts by steers grazing winter wheat forage

Item	Bloated	Nonbloated	*P*-value	SEM
n (sample size)	5	6		
Grazing activity[1]				
Total grazing time (24 h)	9.6	11.4	0.27	1.01
Ruminating (h)	3.2	4.5	0.28	0.74
Idling (h)	10.9	7.8	0.001	0.57
Other activity[2] (min)	26	30	0.8	1.4
Jaw movements (24 h)	52,653	65,872	0.01	2,557
Mastication (h)	1,009	1,172	0.40	123.7
Prehension (h)	1,184	1,571	0.16	162.7
Boli (h)	13.2	14.8	0.67	2.41
Total bouts (24 h)	458	561	0.20	50.5
Ruminating bouts	267	313	0.57	58.4
Grazing bouts	82	150	0.15	29.3

[1]Grazing activities were recorded automatically using mouth sensors to measure their temporal patterns of grazing time, ruminating, and idling behavior over 24 h.

[2]Periods with no jaw movements or unidentified movements were classified as "other activity."

and nonbloated steers; however, the number of prehension jaw movements tended ($P = 0.16$) to be less in bloated than in nonbloated steers (1,184 vs. 1,571 per h). On a daily basis, bloat altered grazing patterns of steers grazing wheat.

Temporal Patterns of Grazing Activity in Bloating and Nonbloating Steers

The temporal patterns of activity exhibited by nonbloated and bloated yearling steers over 24-h periods are shown in Figures 2 and 3. Grazing patterns of nonbloated steers exhibit an up-and-down response with time of day. Grazing time increased from 0600 to 0800 h (sunrise), with maximum grazing activity between 1300 and 1700 h (before sunset) and intermediated activity levels between 1000 and 1200 h. Similar diurnal grazing patterns were observed in bloated steers. The consistency in diurnal pattern of grazing activities over the time is similar to that observed in grazing dairy cows (Gibb et al., 1998). However, the occurrence of grazing activity decreased in steers with bloat from 0400 to 0700 h and 1400 to 1800 h. Overall timing of activities was similar between bloated and nonbloated animals, but the frequency, amplitude, and duration of events led to numerous bloat × time of day × activity interactions ($P < 0.05$).

Intense grazing activities occurred early morning from 0500 to 0800 h in nonbloated steers. However, bloated steers spent less ($P < 0.05$) time in grazing activities from 0400 to 0700 h than did nonbloated animals. Bloat apparently disrupted normal morning grazing patterns and resulted in a 4-h lag ($P < 0.05$) in grazing initiation. The duration of the morning grazing event, therefore, was only 1 h in bloated steers compared with 4 h in nonbloated steers. These results are corroborated by the corresponding depression ($P < 0.01$) in prehension jaw movements in bloated animals compared with nonbloated contemporaries (Figure 3b), which indicates not only less total grazing time but also lower grazing intensity in bloated steers. In both bloated and nonbloated animals, early morning grazing times peaked at 0800 h and did not differ.

Nonbloated steers second and longest grazing sequence occurred from 1000 to 1800 h and displayed alternate hour peaks and valleys in grazing activity, suggesting less intensive grazing from 1000 to 1400 h, which lowered rate of prehension (Figure 3b) over that corresponding time period. Bloated animals also initiated their second grazing sequence at 1000 h, which lasted until 1300 h (Figure 2a). Unlike nonbloated steers, bloated steers did not display the alternate hour peak and valley pattern to begin the second grazing cycle; instead grazing time per hour increased through 1300 h and then precipitously declined by 1400 h and recovered by 1600 h, when it changed to an alternate hour peak and valley grazing activity pattern through 2000 h.

The nighttime grazing sequence in nonbloated cattle lasted from 2000 to 0100 h. Nighttime grazing activity of bloated steers mirrored nonbloated cohorts but was lower in overall magnitude. Initial nighttime prehension rates in nonbloated animals exceeded ($P < 0.05$) those of bloated animals at 2200 h. In contrast, prehension rates in bloated animals tended to exceed ($P = 0.12$) those of nonbloated animals from 2300 to 2400 h, suggesting that bloated animals may have attempted to compensate for less grazing time by increasing biting rate.

Ruminating activity (Figure 2b) generally followed a 3-event sequence in both bloated and nonbloated steers. Major rumination sequences occurred primarily at night from 0200 to 0500 h and 1900 to 2200 h and mid-afternoon at 1400 h, similar to the observations reported by Phillips and Leaver (1985), who investigated with grazing dairy cows. Minor and sporadic rumination events occurred between the grazing bouts during the day. The amplitude and frequency of rumination within peak periods, however, did vary between bloated and nonbloated steers. In early and late rumination sequences, total rumination time was less ($P < 0.01$ to 0.09) in bloated steers. There was a 1-h lag phase in maximum hourly rumination time in bloated steers. The total rumination time and the timing of rumination did not differ for the 1400

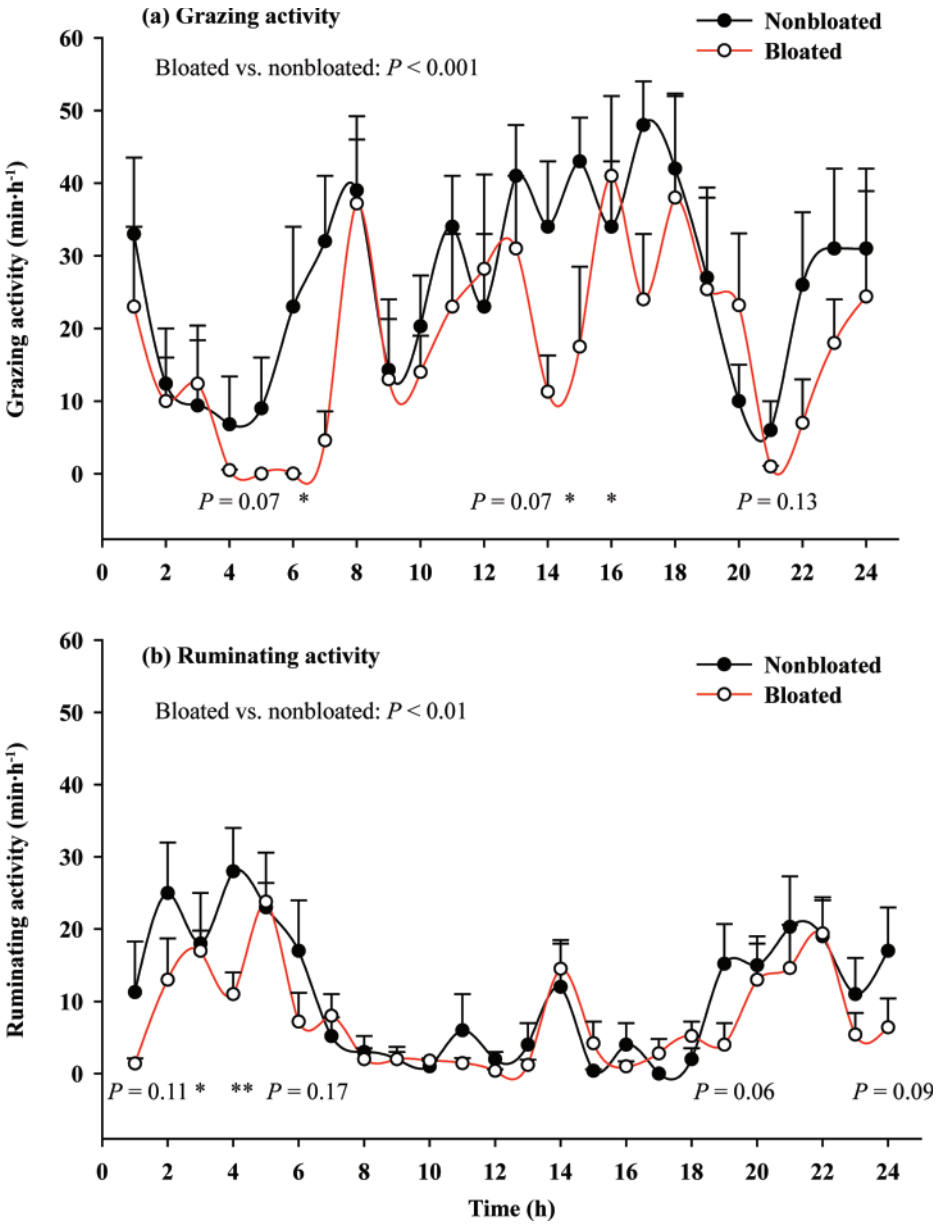

Figure 2. Effect of physiological state (bloated vs. nonbloated) and time of day on grazing activity (a) and ruminating time (b) during experimental period by castrated steers grazing wheat forage. *$P < 0.05$; **$P < 0.01$. Color version available in the online PDF.

h rumination event between bloated and nonbloated animals.

In diurnal idling patterns (Figure 3d), bloated steers spent more time in this activity than did nonbloated steers. Idling activities replaced ($P = 0.08$ to 0.09) grazing activities in bloated steers during early morning and nighttime grazing sequences exhibited in nonbloated steers, but mastication, prehension, and boli activities were generally lower for bloated than for nonbloated steers. This result supports earlier studies (Williams, 1955; Colvin et al., 1958). Williams (1955) reported that primary rumen contractions were similar between bloated and nonbloated cattle, but the secondary contractions were missing. However, the frequency of secondary to primary ruminal-contraction ratios was increased during frothy bloat of cattle fed alfalfa heads (Colvin et al., 1958) and suggested that the patterns of rumination and ruminal functions were in a manner inconsistent with maximum bloat frequency. The present study shows that the steers attempted to maximize intake in the morning (0600 to 0800 h) and before sunset (between 1300 to 1700 h) and spent more time ruminating during the night, but bloated steers exhibited more variable and shorter duration periods of ruminating, mastication, and prehension activities.

The diurnal patterns of prehension activities point to the potential additive effects of bloat on grazing intensity (Figure 3b; $P = 0.06$), as well as grazing time. The lag in prehension rate in bloated steers tended to be greater ($P = 0.14$) than for nonbloated steers. Mastication rates (Figure 3a; $P < 0.03$) at 0100, 0400, and 0600 h in bloated steers were lower than in nonbloated animals. Boli counts from 2300 to 0200 h were lower in bloated than in nonbloated steers. Collectively, these indices of ingestive behavior indicate bloat altered normal grazing and rumination patterns.

Animal susceptibility to bloat has been related to the clearance of small feed particles from the rumen. Frequent bloaters have a slower degradation of feed particle than do nonbloaters (Majak et al., 2003). The present study shows that total grazing time was generally similar between bloated and nonbloated steers, but postingestive activities (ruminating, mastication, and prehension) were concomitantly lower for bloated than for nonbloated steers, suggesting that during bloat, ingested material may be more slowly degraded because of altered ruminal motility and function. Particle-size reduction rate may have been less in bloated steers, thus increasing idling time.

The 16S rDNA PCR technique allowed visualization of microbial population patterns in the rumen of steers related with frothy bloat (Torsvik et al., 1990; Hume et al., 2003). This experiment is the first to record differences in individual rumen bacterial population and grazing patterns between bloated and nonbloated steers grazing wheat forage at differing stages of plant development and

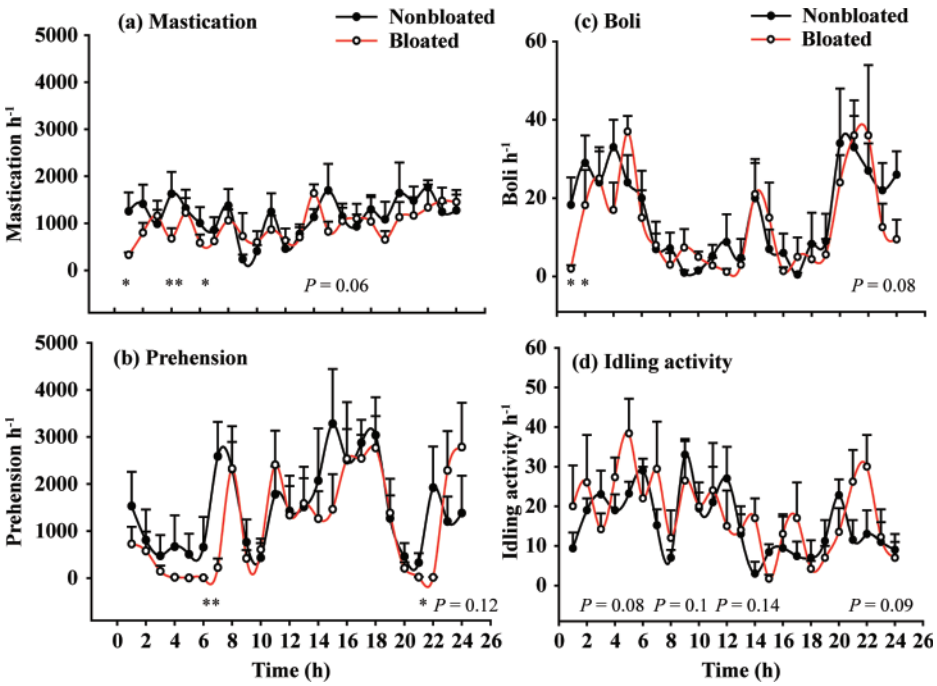

Figure 3. Effect of physiological state (bloated vs. nonbloated) and time of day on mastication (a), prehension (b), boli (c), and idling (d) activities during experimental period by castrated steers grazing wheat forage. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Color version available in the online PDF.

suggests that bloat might be associated with a species-specific change in predominant microflora in the rumen.

IMPLICATIONS

In the present study, bacterial density signals and grazing activity were markedly reduced during the postbloat period. In addition, 2 strains, *S. bovis* and *E. ruminantium*, were not detected in the bloated group in the postbloat period, indicating that bacterial population changes may be caused by bloat severity resulting from a diet-influenced change in the rumen bacterial population. Bloated cattle exhibiting different grazing activity point to the need to develop intervention strategies that require the least amount bloat mitigating compound consumption. Increased idling time in bloated steers suggests that bloat-induced malaise suppresses diurnal grazing patterns. Frothy bloat may partially be associated with the rapid ingestion of large quantities of rapidly fermentable substrate leading to shifts in ruminal microbial populations favorable to the formation of low-gas permeable bio-films to trap rapidly evolved anaerobic fermentation gases. Future studies should focus on bloat-sensitive and bloat-resistant cattle to define their grazing activity with differences, which can shed more light onto the mechanisms of ingestive behavior related to intake of bloat precursors.

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